

22. A method of producing genetically transformed plant cells which are resistant to infection by a plant virus, comprising the steps of:

(a) inserting into the genome of a plant cell a recombinant double-stranded DNA molecule comprising:

(i) a promoter which functions in the plant cell to cause the production of RNA sequences of said plant virus;

(ii) a DNA sequence that causes the production of an RNA sequence, said RNA sequence encoding the coat protein of said plant virus; and

(iii) a 3' flanking sequence; and

(b) recovering transformed plant cells which have increased resistance to infection by said plant virus.

23. A method of claim 22, wherein said DNA sequence is expressed in said transformed plant cells, such that said coat protein is present in said transformed plant cells.

24. A recombinant double stranded DNA molecule comprising in sequence:

(a) a promoter which functions in a plant cell to cause the production of RNA sequences of said plant virus;

(b) a DNA sequence that causes the production of an RNA sequence, said RNA sequence encoding the coat protein of said plant virus; and

(c) a 3' flanking sequence, said molecule being incapable of causing systemic infection in plants.

25. A DNA molecule according to claim 24,
wherein said promoter is heterologous with respect to the coat
protein coding sequence.

26. A plant transformation vector comprising the
DNA molecule according to claim 24.

27. A bacterial cell comprising the plant
transformation vector according to claim 26.

28. A bacterial cell according to claim 27, wherein
said bacterial cell is an *Agrobacterium tumefaciens* cell.

5a4
227

~~29. A transformed plant cell comprising
chromosomal DNA comprised of:~~

- ~~(a) a promoter which functions in plant cells to
cause the production of RNA sequences of a plant virus;~~
- ~~(b) a DNA sequence that causes the production of
an RNA sequence, said RNA sequence encoding the
coat protein of said plant virus; and~~
- ~~(c) a 3' flanking sequence.~~

~~30. A plant cell according to claim 29, said plant
cell exhibiting resistance to said plant virus.~~

5a4
227

~~31. A plant cell according to claim 30, wherein said
DNA sequence is expressed by said plant cell such that said
coat protein is present in said plant cell.~~

32. A plant cell according to claim 29, wherein said
plant virus is tobacco mosaic virus.

33. A plant cell according to claim 32, said cell
being a tobacco cell.

34. A plant cell according to claim 32, said plant cell being a tomato cell.

35. A method according to claim 22, wherein step (a) comprises *Agrobacterium*-mediated insertion of said DNA molecule into a plant cell that is susceptible to infection by *Agrobacterium tumefaciens*.

36. A transformed plant cell of Claim 29, wherein said cell is susceptible to infection by *Agrobacterium tumefaciens*.

sub
av 7

37. A recombinant, double-stranded DNA molecule comprising in sequence:

- (a) a promoter which functions in plant cells to cause the production of RNA sequences of a plant virus;
- (b) a DNA sequence that causes the production of an RNA sequence, said RNA sequence encoding the coat protein of said plant virus; and
- (c) a 3' flanking sequence.

38. A method for production of a virus-resistant dicotyledonous plant, comprising the steps of:

- (a) introducing into the chromosomal genome of a dicotyledonous plant cell a recombinant DNA molecule comprising a viral coat protein structural gene downstream from a plant-expressible promoter;
- (b) recovering transformed plant cells; and
- (c) regenerating virus-resistant dicotyledonous plants from said transformed cells.

39. A method for introducing a trait of virus resistance into a dicotyledonous plant comprising the steps of:

- (a) introducing into cells of a dicotyledonous plant by Agrobacterium-mediated transformation a chimeric DNA sequence which causes the transcription in the cells of a negative strand RNA which is sufficiently complementary in nucleotide sequence to a target RNA strand which is an RNA produced by a virus so that the hybridization of the negative strand DNA to the RNA produced by the virus in vivo will inhibit pathogenic activity of the virus; and
- (b) generating a whole mature plant from said cells; and verifying in said plant the virus resistance trait.

40. A method as claimed in claim 39 wherein the target RNA strand is the RNA of tobacco mosaic virus.

41. A virus resistant morphologically normal dicotyledonous plant comprising:
cells which have in their genome a chimeric DNA sequence which causes the transcription in the cells of a negative strand RNA sufficiently complementary to a target viral RNA strand so that the negative RNA strand will specifically associate with the target-viral RNA strand to inhibit target RNA strand activity in vivo in the cells of the plant, the target viral RNA strand being selected from the group consisting of a portion of the RNA of a virus and the transcription product created in a plant cell by a virus, the chimeric DNA sequence having been inserted into the parental line of the dicotyledonous plant by Agrobacterium-mediated transformation.

42. A tobacco plant comprising
cells which have in their genome a chimeric DNA sequence which causes the transcription in the cells of a negative strand RNA which is sufficiently complementary to a

portion of a target RNA strand so as to associate with the target RNA strand in vivo to inhibit target RNA strand activity in the cells, the target RNA strand selected from the group consisting of a RNA product of viral infection of the cell, and a viral RNA itself, the chimeric DNA sequence having been inserted into the parental line of the plant by Agrobacterium-mediated transformation.

43. A tobacco plant as claimed in claim 42 wherein the target RNA strand is the coat protein in RNA of tobacco mosaic virus.

44. A tobacco plant as claimed in claim 43 wherein the target RNA strand is the RNA of tobacco mosaic virus.

45. Seeds of a plant as claimed in Claim 41.

46. Seeds of a tobacco plant as claimed in Claim 42.

47. A virus-resistant dicotyledonous plant comprising in its chromosomal genome a DNA construct comprising a plant-expressible promoter-upstream from a DNA-sequence which causes the transcription of a negative strand RNA complementary to viral RNA.

48. A virus-resistant plant comprising in its genome a DNA construct comprising a plant-expressible promoter upstream from a DNA sequence which causes the transcription of a negative strand RNA complementary to viral RNA, the DNA sequence introduced into a parent of the plant through Agrobacterium-mediated plant transformation.

49. A virus-resistant plant comprising in its genome a DNA construct comprising a promoter normally operable in

plant cells upstream from a DNA sequence which causes the transcription of a negative strand RNA which is complementary to viral RNA and which inhibits pathogenesis by the virus.

50. A virus-resistant dicotyledonous plant comprising in its genome a DNA construct comprising a promoter normally operable in plant cells upstream from a DNA sequence which causes the transcription of a negative strand RNA which is complementary to viral RNA and which inhibits pathogenesis by the virus.

51. A virus-resistant plant comprising in its genome a DNA construct comprising a promoter normally operable in plant cells upstream from a DNA sequence which causes the transcription of a negative strand RNA which is complementary to viral RNA and which inhibits pathogenesis by the virus, wherein the DNA construct was introduced into a parent by the plant by Agrobacterium-mediated plant transformation.

52. A method for producing a virus-resistant dicotyledonous plant comprising the steps of:

- (a) introducing into the chromosomal genome of a dicotyledonous plant cell a recombinant DNA molecule comprising a plant-expressible promoter upstream from a DNA sequence which causes the transcription of a negative strand RNA complementary to viral RNA;
- (b) recovering transformed plant cells; and
- (c) regenerating virus-resistant dicotyledonous plants from said transformed cells.

53. A method for producing a virus-resistant plant comprising the steps of:

- (a) introducing into the genome of a plant cell by Agrobacterium-mediated transformation a recombinant

DNA molecule comprising a promoter normally operable in plant cells upstream from a DNA sequence which causes the transcription of a negative strand RNA complementary to viral RNA;

(b) recovering transformed plant cells; and

(c) regenerating virus-resistant plants from said transformed cells.

54. A method for producing a virus-resistant plant comprising the steps of:

(a) introducing into the genome of a plant cell a recombinant DNA molecule comprising a promoter normally operable in plant cells upstream from a DNA coding sequence which causes the transcription of a negative strand RNA sufficiently complementary to a viral RNA to inhibit pathogenesis by the virus;

(b) recovering transformed plant cells; and

(c) regenerating virus-resistant dicotyledonous plants from said transformed cells.

55. A method for producing a virus-resistant plant comprising the steps of:

(a) introducing into the genome of a plant cell by Agrobacterium-mediated transformation a recombinant DNA molecule comprising a promoter normally operable in plant cells upstream from a DNA coding sequence which causes the transcription of a negative strand RNA sufficiently complementary to a viral RNA to inhibit pathogenesis by the virus;

(b) recovering transformed plant cells; and

(c) regenerating virus-resistant plants from said transformed cells.

56. A chimeric DNA sequence comprising a promoter normally operable in plant cells and DNA sequence coding for the transcription of a negative strand RNA which is sufficiently complementary in nucleotide sequence to a target RNA strand produced by a virus so that expression of the chimeric DNA sequence in plant cells will inhibit pathogenic activity of the virus.

57. A plant cell comprising in its genome the chimeric DNA sequence of Claim 56.

58. A DNA construct comprising a plant-expressible promoter upstream from a DNA sequence which causes the transcription of a negative strand RNA complementary to viral RNA, wherein the promoter is capable of causing transcription of said DNA sequence in a plant cell.

59. A virus-resistant dicotyledonous plant cell comprising the DNA construct of Claim 58.

60. A method of producing virus resistant plants comprising:

introducing into a plant cell a DNA coding for a gene, or fragment thereof, of the virus which when introduced into plant cells inhibits pathogenesis by the virus;
recovering transformed plant cells; and
regenerating virus resistant plants from said transformed cells.

525
257 61. The method according to claim 60, wherein said plant is a tomato plant.

62. The method according to claim 60, wherein said plant is cotton.

63. The method according to claim 60, wherein said plant is potato and said virus is potato virus X.

64. The method according to claim 60, wherein said plant is potato and said virus is potato virus Y.

65. The method according to claim 60, wherein said gene or fragment thereof is a coat protein gene or a fragment thereof.

sub 5
a6 } 66. The method according to claim 60, wherein the DNA coding for the gene, or fragment thereof, of the virus is operably linked with a promoter which causes expression of a product sufficient to inhibit pathogenesis by the virus.

67. The method according to claim 60, wherein the DNA is in the sense direction.

68. The method according to claim 60, wherein the DNA is in the anti-sense direction.

sub 5
a7 } 69. The method according to claim 60, wherein the DNA sequence encodes a viral replicase gene or a fragment thereof.

70. The method according to claim 60, wherein the DNA encodes a viral replicase binding site.

71. The method according to claim 60, wherein the DNA encodes a viral coat protein or a fragment thereof.

sub 5
a8 } 72. The method according to claim 60, wherein said virus is selected from the group consisting of alfalfa mosaic

virus, brome mosaic virus, barley yellow dwarf virus, beet yellows virus, cucumber mosaic virus, lettuce necrotic yellow virus, maize chlorotic dwarf virus, pea enation virus, potato virus S, potato virus X, potato virus Y, southern bean mosaic virus, tomato ringspot virus, tobacco ringspot virus, tobacco mosaic virus, tobacco streak virus, turnip yellow mosaic virus, and wound tumor virus.

73. The method according to claim 72, wherein said virus is selected from the group consisting of tobacco mosaic virus, cucumber mosaic virus, alfalfa mosaic virus, and tobacco ringspot virus.

74. A recombinant DNA molecule comprising: a promoter which is functional in plant cells; and a DNA sequence which encodes a protein native to a virus operably linked to the promoter, wherein said recombinant DNA molecule when introduced into the plant cells will prevent the propagation of the virus.

75. The recombinant DNA molecule according to claim 74, wherein the DNA sequence is in the sense direction.

76. The recombinant DNA molecule according to claim 74, wherein the DNA sequence is in the anti-sense direction.

SUB 99) 77. The recombinant DNA molecule according to claim 74, wherein the DNA sequence encodes a viral replicase gene or a fragment thereof.

78. The recombinant DNA molecule according to claim 74, wherein the DNA sequence encodes a viral replicase binding site.

79. The recombinant DNA molecule according to claim 74, wherein the DNA sequence encodes a viral coat protein or a fragment thereof.

sub
a 10 } 80. The recombinant DNA molecule according to claim 74, wherein said virus is selected from the group consisting of alfalfa mosaic virus, brome mosaic virus, barley yellow dwarf virus, beet yellows virus, cucumber mosaic virus, lettuce necrotic yellow virus, maize chlorotic dwarf virus, pea enation virus, potato virus S, potato virus X, potato virus Y, southern bean mosaic virus, tomato ringspot virus, tobacco ringspot virus, tobacco mosaic virus, tobacco streak virus, turnip yellow mosaic virus, and wound tumor virus.

81. The recombinant DNA molecule according to claim 80, wherein said virus is selected from the group consisting of tobacco mosaic virus, cucumber mosaic virus, alfalfa mosaic virus, and tobacco ringspot virus.

sub
a 11 } 82. A vector containing the recombinant DNA molecule according to claim 74.

83. The vector according to claim 82, wherein said vector is an *Agrobacterium tumefaciens* plasmid.

84. A host cell transformed with the vector according to claim 82.

85. The host cell according to claim 84, wherein the host cell is a bacterium.

86. The host cell according to claim 85, wherein the bacterium is *Agrobacterium tumefaciens*.

87. The host cell according to claim 84, wherein the host cell is a plant cell.

88. The host cell according to claim 87, wherein the plant cell is dicotyledonous

89. The host cell according to claim 87, wherein the plant cell is monocotyledonous

90. A method of producing virus resistant plants, comprising:
introducing into a plant cell a DNA coding for a gene, or fragment thereof, of the virus which when introduced into plant cells inhibits pathogenesis by the virus;
recovering transformed plant cells; and
regenerating virus resistant plants from said transformed cells.

91. The method according to claim 90, wherein said plant is a tomato plant.

92. The method according to claim 90, wherein said plant is cotton.

93. The method according to claim 90, wherein said plant is potato and said virus is potato virus X.

94. The method according to claim 90, wherein said plant is potato and said virus is potato virus Y.

95. The method according to claim 90, wherein said gene or fragment thereof is a coat protein gene or a fragment thereof.

96. The method according to claim 90, wherein the DNA coding for the gene, or fragment thereof, of the virus is operably linked with a promoter which causes expression of a product sufficient to inhibit pathogenesis by the virus.

97. The method according to claim 90, wherein the DNA is in the sense direction.

98. The method according to claim 90, wherein the DNA is in the anti-sense direction.

99. The method according to claim 90, wherein the DNA sequence encodes a viral replicase gene or a fragment thereof.

100. The method according to claim 90, wherein the DNA encodes a viral replicase binding site.

101. The method according to claim 90, wherein the DNA encodes a viral coat protein or a fragment thereof.

102. The method according to claim 90, wherein said virus is selected from the group consisting of alfalfa mosaic virus, brome mosaic virus, barley yellow dwarf virus, beet yellows virus, cucumber mosaic virus, lettuce necrotic yellow virus, maize chlorotic dwarf virus, pea enation virus, potato virus S, potato virus X, potato virus Y, southern bean mosaic virus, tomato ringspot virus, tobacco ringspot virus, tobacco mosaic virus, tobacco streak virus, turnip yellow mosaic virus, and wound tumor virus.

103. The method according to claim 102, wherein said virus is selected from the group consisting of tobacco

mosaic virus, cucumber mosaic virus, alfalfa mosaic virus, and tobacco ringspot virus.

104. A virus resistant plant comprising a recombinant DNA molecule having a gene, or fragment thereof, isolated from the virus, wherein said gene, or fragment thereof, when introduced into plant cells inhibits pathogenesis by the virus.

sub
a12

105. The virus resistant plant according to claim 104, wherein the gene, or fragment thereof is maintained within the plant genome.

106. The virus resistant plant according to claim 104, wherein the gene, or fragment thereof is maintained on a vector capable of replicating in the host.

107. The virus resistant plant according to claim 104, wherein the plant is dicotyledonous

108. The virus resistant plant according to claim 104, wherein the plant is monocotyledonous

109. The virus resistant plant according to claim 104, wherein said plant is a tomato plant.

110. The virus resistant plant according to claim 104, wherein said plant is cotton.

111. The virus resistant plant according to claim 104, wherein said plant is potato and said virus is potato virus X.

112. The virus resistant plant according to claim 104, wherein said plant is potato and said virus is potato virus Y.

113. The virus resistant plant according to claim 104, wherein said gene or fragment thereof is a coat protein gene or a fragment thereof.

sub
aug 7 114. The virus resistant plant according to claim 104, wherein the DNA coding for the gene, or fragment thereof, of the virus is operably linked with a promoter which causes expression of a product sufficient to inhibit pathogenesis by the virus.

115. The virus resistant plant according to claim 104, wherein the DNA is in the sense direction.

116. The virus resistant plant according to claim 104, wherein the DNA is in the anti-sense direction.

sub
aug 7 117. The virus resistant plant according to claim 104, wherein the DNA sequence encodes a viral replicase gene or a fragment thereof.

118. The virus resistant plant according to claim 104, wherein the DNA encodes a viral replicase binding site.

119. The virus resistant plant according to claim 104, wherein the DNA encodes a viral coat protein or a fragment thereof.

sub
aug 7 120. The virus resistant plant according to claim 119, wherein said virus is selected from the group consisting of alfalfa mosaic virus, brome mosaic virus, barley yellow dwarf virus, beet yellows virus, cucumber mosaic virus, lettuce necrotic yellow virus, maize chlorotic dwarf virus, pea enation virus, potato virus S, potato virus X, potato virus Y, southern bean mosaic virus, tomato ringspot virus, tobacco ringspot

virus, tobacco mosaic virus, tobacco streak virus, turnip yellow mosaic virus, and wound tumor virus.

121. The virus resistant plant according to claim 104, wherein said virus is selected from the group consisting of tobacco mosaic virus, cucumber mosaic virus, alfalfa mosaic virus, and tobacco ringspot virus.

122. A method of making a host cells resistant to a virus for the host, comprising:

- a) isolating DNA coding for a gene, or fragment thereof, of said virus;
- b) operably linking said DNA, or fragment thereof, within an expression vector; and
- c) transforming said host cells with said expression vector.

123. The method according to claim 122, wherein said DNA or fragment thereof is expressed in the sense direction.

124. The method according to claim 122, wherein said DNA or fragment thereof is expressed in the anti-sense direction.

sub
ack } 125. The method according to claim 122, wherein said gene or fragment thereof is a viral replicase gene or a fragment thereof.

126. The method according to claim 122, wherein said gene or fragment thereof contains a binding site for a replicase enzyme.

~~127. The method according to claim 122, wherein said gene or fragment thereof is a coat protein gene or a fragment thereof.~~

~~128. A method of making plant cells or plant tissue resistant to infection by one or more virus, comprising:~~

- ~~a) isolating DNA coding for a gene, or fragment thereof, of said virus;~~
- ~~b) operably linking said DNA, or fragment thereof, within an expression vector; and~~
- ~~c) transforming said plant cells or plant tissue with said expression vector.~~

~~129. The method according to claim 128, wherein said DNA or fragment thereof is expressed in the sense direction.~~

~~130. The method according to claim 128, wherein said DNA or fragment thereof is expressed in the anti-sense direction.~~

Sub 9.17 > ~~131. The method according to claim 128, wherein said gene or fragment thereof is a viral replicase gene or a fragment thereof.~~

~~132. The method according to claim 128, wherein said gene or fragment thereof contains a binding site for a replicase enzyme.~~

~~133. The method according to claim 128, wherein said gene or fragment thereof is a coat protein gene or a fragment thereof.~~